Nodal follicular lymphoma without complete follicular dendritic cell networks is related to localized clinical stage

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Follicular lymphoma is the most common low-grade lymphoma and it frequently presents with a systemic disease, often showing advanced clinical stage (III/IV). The lymphoma cells are usually growing associated with follicular dendritic cell (FDC) networks. Abnormal FDC networks have been reported in duodenal follicular lymphoma, in which cases exhibit lower clinical stages than the nodal cases. In the present study, we analyzed the FDC network distribution pattern of 242 nodal follicular lymphomas by immunohistochemistry. Out of the 242 cases, 27 cases (11%) demonstrated an atypical pattern of FDC networks, in which the CD21 staining totally or partially disappeared in the neoplastic follicles. Furthermore, we compared the clinical data of these 27 cases and 58 typical FDC network cases of follicular lymphoma. We found that in the typical cases, 52 out of 58 patients (90%) showed advanced clinical stage (III or IV), whereas 10 of 27 (37%) atypical FDC network cases showed localized clinical stage (I or II) ($P < 0.01$). In conclusion, nodal follicular lymphoma with total loss or partially disrupted FDC networks therefore show a lower clinical stage.

Key words: CD21, clinical stage, follicular dendritic cell, follicular lymphoma, Ki-67 labeling index

Follicular lymphoma (FL) is a neoplasm composed of follicle center B cells which usually shows a follicular pattern.[1] The majority of FLs originate from lymph node, and roughly 70% of the patients are diagnosed as advanced clinical stages (III or IV). Bone marrow involvement is found in up to 50% of the cases. In the majority (70%–95%) of FLs with histological grade 1, 2, and 3A, a characteristic chromosomal translocation of t(14; 18)(q32; q21) can be detected.[1–3]

We have reported that FL frequently originates from duodenum,[4] and most of them are at lower clinical stages than the nodal cases.[4,5] Although follicular dendritic cell (FDC) networks densely occupied the neoplastic follicle areas in most of the nodal FL, we found that in duodenal FL, FDC networks were frequently distributed to the periphery of the neoplastic follicles, and its clinical features similar to mucosa-associated lymphoid tissue (MALT) lymphoma.[6] As a matter of fact, this duodenal-like FDC pattern was occasionally observed in a small fraction of cases of nodal FL.

Considering this finding, we examined the FDC networks patterns in cases of nodal FLs by immunostaining for FDC marker, CD21,[7] and investigated the clinical data of relevant patients, thereby evaluating whether the characteristic distribution of FDC networks related to the disease progression.

MATERIALS AND METHODS

Case selection and clinical data

Two hundred and forty-two cases of nodal FL were retrieved from the surgical pathology consultation files of the Department of Pathology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan between 1995 and 2009.

All samples examined in the present study were lymph node biopsy sections. Histological sections and immunohistochemistry were reviewed to confirm the diagnosis. The neoplastic follicles displayed a CD10 positive and/or Bcl2 positive to distinguish from other low-grade B cell lymphoma. Cases of FL grade 3B were not included in the study because these tumors may have different biologic behavior.[1,8] Clinical data were kindly provided by the referring clinicians, or obtained from medical records of patients. All data and samples from patients were collected with their informed consent.

Immunohistochemistry

All immunohistochemistry was performed on paraffin sections using standard antigen retrieval protocols, an automated immunostainer (Ventana Medical System, Tucson, AZ, USA). The primary antibodies used were: CD10 (56C6, 1:100, Leica, Newcastle, UK), Bcl2 (3.1, 1:100, Leica), Ki-67 (MIB-1, 1:5000; Novocastra, Newcastle, UK) and CD21 (1F8, 1:20; DAKO, Glostrup, Denmark).[5]

Ki-67 labeling index was evaluated semiquantitatively using the average estimated percentage of positive cells in 10 recorded high-power fields. All data and samples from patients were collected with their informed consent.

Statistical analysis

Differences of clinical stage data between the study groups was tested on their statistical significance using Fisher's exact test. Comparisons among mean values were performed using *t*-test. SPSS version 14.0 (SPSS, Chicago, IL, USA) was used to analyze the data.

RESULTS

CD21 expression pattern

The distribution of FDC networks of 242 cases, which were diagnosed as nodal FL were examined by immunohistochemistry, and the staining patterns of FDC expression for CD21 are shown in Fig. 1. Two hundred and fifteen of 242 (89%) cases showed typical (nodal) FDC networks pattern (Fig. 1a), in which the FDC were densely found within neoplastic follicle areas. In contrast, the remaining 27 (11%) cases showed several types of atypical FDC pattern, which were identified as partial or complete loss of FDC antigen CD21 expression in neoplastic follicle areas. According to the characteristic distribution of FDC networks, we further divided these 27 cases into three patterns: FDC networks were absent in most of the neoplastic follicle areas and was referred to as vanished pattern (17 cases, 63%). FDC distributed in the periphery of the neoplastic follicles areas was referred to as duodenal pattern (three cases, 11%). And finally, marginal pattern (seven cases, 26%) in which FDC meshworks occupied more than half of the neoplastic follicle areas, but were absent in the center of the tumor follicles. (Fig. 1b–d)

Clinical data and statistics analysis

The study group consisted of 27 cases (16 male, 11 female; age range, 39–84; median, 59.0) with an atypical FDC pattern. For control group, clinical data from 58 cases with typical FDC pattern were selected based on the available clinical records (34 male, 24 female; age range, 32–85; average, 58.1). All the clinicopathologic data are summarized in Table 1. There was no significant difference in the histological grade between study and control group.

Clinical stages in the study group of 27 cases with the atypical FDC pattern, were stage I (four cases), II (six cases), III (11 cases) and IV (six cases), resulting in a systemic disease stage (III or IV) in 17 of 27 cases (63%). In the control group with typical FDC pattern, 90% of the patients (52 of 58 cases) suffered from a systemic disease. There were no cases in clinical stage I, and only six of 58 cases (10%) showed localized clinical stage II. The difference between the study and control group was statistically significant ($P < 0.01$). We further compared the three sub-classified atypical FDC pattern cases with the control group separately. Interestingly, the vanished and duodenal FDC pattern cases showed a high rate of localized clinical stage ($P < 0.05$), but there was no statistically significant difference in the cases with marginal FDC pattern (Table 2).

Ki-67 labeling index

Cases with histological grade 1 were selected from the study group (16 cases) and the control group (30 cases) for immunostaining with Ki-67. The Ki-67 labeling index ranged from 4% to 15% in the study group, and 2% to 13% in the control group (data not shown). There was no significance between the study group and the control group.

DISCUSSION

Follicular lymphoma is a representative indolent B-cell lymphoma; it grows very slowly and most patients show rather long clinical course. Although clinically indolent, these lymphoma patients have widespread disease at diagnosis, including peripheral and central (abdominal and thoracic) lymphadenopathy and splenomegaly. Histologically, it is characterized by nodular proliferation of neoplastic B cells.[1–3]

The FDC networks present in the reactive and neoplastic germinal centers (GC) play important roles for providing microenviromental conditions to GC B cells: their presence facilitates the survival and proliferation of GC lymphocytes.[7–12] Moreover, FDCs are important to prevent apoptosis of FL cells; this effect is related to the expression of activated markers such as CD54 (ICAM-1), CD106 (VCAM-1) and CD40. FDCs in FL express a variable spectrum of the differentiation markers, ranging from a pattern similar to reactive follicles with expression of the germinal center specific chemokine CXCL13, CD23, CD21 and CD35 to a more undifferentiated status with loss of CD23, CD21 and CD35 but still expressing CXCL13. Disruption of these patterns suggest related to the progression and transformation of FL.[10,13]

In FL lymphoma, structural alterations such as an atypical shape or expansion of the FDC networks are frequently detectable both in nodal and extranodal sites.[14,15] In the present study, we found that the occurrence of the atypical FDC pattern with loss of CD21 FDC antigen was significantly related to localized clinical stage. This phenomenon is similar to duodenal FL. The duodenal FL usually lack FDC networks and showed localized clinical stage,[5,6] and this FDC pattern is somewhat similar to the follicular colonization of mucosa-associated tissue (MALT) lymphomas, in which marginal zone B-cell lymphoma cells infiltrate the non-neoplastic GC and destroy FDC networks.[6] Therefore we considered duodenal FL share some common characteristics with MALT lymphoma.[5,6] It could be concluded that loss of FDC in neoplastic follicles of nodal FL may show some common features in pathologic process with duodenal, and exhibit a similar limited clinical stage.

Follicular dendritic cells can be detected among the reactive bystander cells of the tumor in non-Hodgkin lymphomas. It has been suggested that FDC in FL are newly generated during lymphoma expansion, whereas the FDC are considered to represent remnants of the germinal center that have been colonized by neoplastic cells in marginal zone lymphoma.[14,16] If the FDC are newly generated in FL, the pre-forming reactive germinal centers for the lymphoma neoplastic cells proliferation are needed. Microdissection from two follicles in one case for PCR of IgVH genes were illustrated that besides the tumor band being present in all lanes, in addition, other bands were apparent, some of which were apparent, some of which were shared between microdissections from the same follicle. These findings suggest an oligoclonal GC population.[17] Similar studies using genealogical trees for the V_H and the V_L gene rearrangements were constructed to analyze the clonal relationship among individual cells of distinct follicles of each case. The observation that the neoplastic cells migration depend on the follicular microenvironment for their clonal expansion.[18] So total or partial lack of FDC networks cause a low-functional germinal center to be formed and inefficient co-stimulation with neoplastic cells. We further compared the three subclassified atypical FDC pattern cases with the control group separately. Interestingly, the vanished FDC pattern and duodenal FDC pattern cases show a high rate of limited clinical stage ($P < 0.05$), but there was no statistically significant difference in the cases with marginal FDC pattern. This further testified our finding that the neoplastic cells expansion prefers a FDC network relative to intact and FLs growth exhibits a more progressing process and higher clinical stage.

Undifferentiated stroma in FL may result from failure to recruit FDCs, or to provide signals for complete FDC maturation, or as a result of a progressive loss of FDC differentiation in preformed GCs that have been colonized by lymphoma.[15] Although the vanished FDC pattern cases showed a high rate of limited clinical stage in contrast to typical cases, 11 of 17 cases (65%) still showed a more aggressive clinical stage. Previous observation by Dogan *et al.*[19] revealed that the interfollicular tumor component in FL cases usually has a much lower mitotic

activity than the follicular component. This suggests that neoplastic cells may be aggregating in interfollicular areas in contrast to diffuse growth forming descrete areas, indicating that extrafollicular proliferation was occurring.[10,20,21] In our study, limited stage cases of the vanished group showed a partial nodularity pattern but a lack of FDC differentiation. By contrast, a dominantly diffuse pattern was found in the rest of the vanished group, in which it showed a progressive clinical stage (data not shown). The changes of FDC differentiation in the former group may be caused by antigen loss, followed by loss of FDC maturation. In the latter group, lymphoma growth may have shifted to a FDC-free growth pattern. Further study should be established on this view.

There was no significant correlation between the FDC pattern and the Ki-67 labeling index, which may be due to the low Ki-67 labeling index in each group with histological grade 1. Therefore, FDC may relate to the migration of lymphoma cells rather than their proliferation activity.

In conclusion, our data revealed the atypical FDC pattern of FLs, which was found in 11% of examined FLs, significantly showed localized clinical stage (I or II) more than typical FDC pattern of FLs and the FDC pattern suggest that related to the spread of the disease.

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Figure 1 Immunohistochemical stain of CD21 in nodal follicular lymphomas. (**a**) Typical pattern of follicular dendritic cell (FDC) expressing CD21. FDC networks are densely found in neoplastic follicle areas; (**b**) Vanished pattern. FDC networks were absent in most of the neoplastic follicle areas; (**c**) Duodenal pattern. FDC networks were distributed in the periphery of the neoplastic follicles areas; (**d**) marginal pattern. FDC networks occupied more than half of the neoplastic follicle areas, but were absent in the center of the neoplastic follicle areas.

Table 1 Clinicopathologic data of nodal follicular lymphomas

	Control group (typical FDC pattern)	Study group (atypical FDC pattern)	
	n = 58 (%)	*n* = 27 (%)	*P-value*
Average age (range)	58.1 (32–85)	59.0 (39–84)	
Gender (M/F)	34/24	16/11	
Histological grade (*n* = 85)			
1	35 (60%)	16 (59%)	0.999
2	10 (17%)	9 (33%)	0.285
3A	6 (10%)	2 (7%)	0.999
NOS	5 (9%)	0	0.316
With diffuse areas	2 (3%)	0	0.999

P-value was determined by Fisher's exact test.
FDC, follicular dendritic cell; NOS, not other specific.

Table 2 Number (%) of patients with clinical stages according to follicular dendritic cell (FDC) network features in the neoplastic follicle areas

	Control group (typical FDC pattern)	Study group (atypical FDC pattern)	Cases with vanished pattern of FDC network	Cases with duodenal pattern of FDC network	Cases with marginal pattern of FDC network
	n = 58 (%)	*n* = 27 (%)	*n* = 17 (%)	*n* = 3 (%)	*n* = 7 (%)
Stage I	0 (0)	4 (15)	3 (18)	1	0
Stage II	6 (10)	6 (22)	3 (18)	1	2
Localized disease (Stage I/II)	6 (10)	10 (37)	6 (35)	2	2
Stage III	26 (45)	11 (41)	7 (41)	1	3
Stage IV	26 (45)	6 (22)	4 (24)	0	2
Systemic disease (Stage III/IV)	52 (90)	17 (63)	11 (65)	1	5
P-value		0.0062	0.023	0.043	0.203

P-value was determined by Fisher's exact test.
The difference of clinical stages between the study group and the control group was statistically significant ($P < 0.05$).